

The replacement paragraph above includes changes indicated by the marked-up version below.

This application is a continuation of U.S. Serial Number 08/674,509, filed July 2, 1996, which is a continuation-in-part of U.S.S.N. Serial Number 08/460,600, filed June 5, 1995, which is a continuation-in-part of U.S.S.N. Serial Number 08/435,093, filed May 4, 1995, which is a continuation-in-part of U.S.S.N. Serial Number 08/356,060, filed December 14, 1994, which is a continuation-in-part of U.S.S.N. Serial Number 08/227,371 filed December 30, 1993, the teachings of each specification being of which are hereby incorporated by reference herein.

In the claims:

For the convenience of the Examiner, all claims being examined, whether or not amended, are presented below.

✓ Please cancel claims 2, 8-16, 28, 37-41, and 50-62 without prejudice.

a2 1. (Amended) An assay for identifying compounds that mimic the bioactivity of a *hedgehog* protein, comprising:

- (a) forming a reaction mixture including:
 - (i) a naturally occurring *patched* receptor; and
 - (ii) a test compound; and
- (b) detecting activation of the *hedgehog* pathway;

wherein a statistically significant change in the activation of the *hedgehog* pathway in the presence of the test compound, relative to the activation in the absence of the test compound, indicates a *hedgehog*-mimicking activity for the test compound.

a3 3. (Amended) The assay of claim 1, wherein the reaction mixture comprises a cell including a heterologous nucleic acid recombinantly expressing the *patched* receptor.

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4. (Amended) The assay of claim 1, wherein detecting activation of the *hedgehog* pathway comprises observing a phenotype of a cell in the presence and absence of the test compound.

5. (Amended) The assay of claim 3, wherein detecting activation of the *hedgehog* pathway comprises detecting a change in the level of an intracellular second messenger responsive to signaling by the *patched* polypeptide.

6. (Amended) The assay of claim 3, wherein detecting activation of the *hedgehog* pathway comprises detecting a change in the level of expression of a gene controlled by a transcriptional regulatory sequence responsive to signaling by the *patched* polypeptide.

7. (Amended) The assay of claim 3, wherein the recombinant cell substantially lacks expression of an endogenous *patched* receptor.

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17. (Amended) An assay for screening test compounds to identify agents that mimic a bioactivity of a *hedgehog* protein, comprising:

- i. providing a cell expressing a *hedgehog* receptor, wherein said *hedgehog* receptor binds a naturally occurring *hedgehog* polypeptide;
- ii. contacting the cell with a test compound; and
- iii. detecting activation of the *hedgehog* receptor,

wherein a statistically significant change in the level of activation of the *hedgehog* receptor is indicative of an agent that mimics a bioactivity of a *hedgehog* protein.

18. (Amended) The assay of claim 17, wherein the activation of the *hedgehog* receptor is detected by detecting a change in phenotype of the cell relative to in the absence of the test compound.

19. The assay of claim 17, wherein the change in phenotype is detected by detecting gain or loss of expression of a cell-type specific marker.

20. (Amended) The assay of claim 17, wherein the receptor transduces a signal in the cell which is sensitive to *hedgehog* binding, and the cell further comprises a reporter gene construct

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Costal-2
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comprising a reporter gene in operable linkage with a transcriptional regulatory sequence sensitive to intracellular signals transduced by activation of the *hedgehog* receptor, expression of the reporter gene providing a detectable signal for detecting activation of the *hedgehog* receptor.

21. (Amended) The assay of claims 20 or 33, wherein the reporter gene encodes a gene product that gives rise to a detectable signal selected from color, fluorescence, luminescence, cell viability, relief of a cell nutritional requirement, cell growth, and drug resistance.

22. (Amended) The assay of claim 21, wherein the reporter gene encodes a gene product selected from chloramphenicol acetyl transferase, luciferase, betagalactosidase, and alkaline phosphatase.

23. (Amended) The assay of claim 20, wherein the reporter gene includes a transcriptional regulatory sequence of a gene selected from a *GLI* gene and *patched* gene.

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24. (Amended) The assay of claim 17, wherein the receptor transduces a signal in the cell which is sensitive to *hedgehog* binding, and activation of the *hedgehog* receptor is detected by detecting change in a level of an intracellular second messenger responsive to signaling by the receptor.

25. (Amended) The assay of claim 24, wherein the activation of the *hedgehog* receptor is detected by changes in intracellular protein phosphorylation.

26. The assay of claim 17, wherein the receptor is a *patched* receptor.

27. (Amended) The assay of claim 17 or 26, wherein the cell further comprises a heterologous gene construct encoding the receptor.

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29. The assay of claim 17, wherein the cell further comprises one or more heterologous gene constructs encoding *costal-2*, *fused* and/or *smoothened* genes, or homologs thereof.

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30. (Amended) An assay for screening test compounds to identify agents that activate a naturally occurring mammalian *patched* receptor, comprising:

- 50 C3
- i. providing a cell having a recombinant expression vector encoding a naturally occurring mammalian *patched* receptor;
 - ii. contacting the cell with a test compound under conditions whereunder the *patched* protein is expressed; and
 - iii. detecting an effect, if any, of the test compound on signal transduction by the *patched* protein,

wherein a statistically significant change in the signal transduction of the *patched* receptor in the presence of the test compound, relative to in the absence of the test compound or the *patched* receptor, is indicative of an agent that modulates the activity of *patched* receptor.

31. (Amended) The assay of claim 30, wherein the signal transduction by the *patched* receptor is detected by detecting a change in phenotype of the cell relative to in the absence of the test compound.

32. (Amended) The assay of claim 30, wherein the cell is a human cell.

33. (Amended) The assay of claim 30, wherein the cell further comprises a reporter gene construct comprising a reporter gene in operable linkage with a transcriptional regulatory sequence sensitive to intracellular signals transduced by interaction of a *hedgehog* polypeptide with the *patched* receptor, expression of the reporter gene providing a detectable signal for detecting signal transduction by the *patched* receptor.

34. (Amended) The assay of claims 1, 15, 26 or 30, wherein the *patched* receptor is of vertebrate origin.

35. (Amended) The assay of claim 34, wherein the *patched* receptor is a mammalian *patched* receptor.

36. (Amended) The assay of claim 35, wherein the *patched* receptor is a human *patched* receptor.

94 42. (Amended) The assay of any of claims 3, 17 or 30, wherein the cell is a metazoan cell.

43. (Amended) The assay of claim 42, wherein the cell is a mammalian cell.

44. (Amended) The assay of claim 42, wherein the cell is an insect cell.

45. (Amended) The assay of any of claims 3, 17, or 30, wherein the cell is a oocyte.

46. (Amended) The assay of any of claims 3, 17, or 30, wherein the cell is a yeast cell.

47. (Amended) The assay of claims 1, 8, 17, or 30, wherein the steps of the assay are repeated for a variegated library of at least 100 different test compounds.

48. (Amended) The assay of claims 1, 8, 17 or 30, wherein the test compound is selected from small organic molecules; and natural product extracts.

49. (Amended) The assay of claims 1, 8, 17 or 30, further comprising preparing a pharmaceutical preparation of one or more compounds identified.

Please add the following new claims:

97 63. (New) A method for identifying *hedgehog* agonists, comprising:

contacting a test agent with cells expressing a *patched* receptor, wherein said cells undergo a detectable response when contacted with a naturally occurring *hedgehog* protein, which response is dependent on expression of the *patched* receptor; and comparing the response of said cells to the test agent with the response of similar cells to a naturally occurring *hedgehog* protein.

64. (New) A method of claim 63, wherein said detectable response comprises expression of a gene controlled by a transcriptional regulatory sequence responsive to *patched*-mediated *hedgehog* signaling.

65. (New) A method of claim 64, wherein said detectable response comprises expression of secondary signaling molecules selected from Bmp-2, Bmp-4, and Fgf-4.

66. (New) A method of claim 63, wherein said cells are transfected to express a recombinant form of the *patched* receptor.

67. (New) A method of claim 63 or 66, wherein said cells are eukaryotic.

68. (New) A method of claim 63, 66, or 67, wherein said cells are vertebrate cells.

69. (New) A method of claim 63, 66, or 67, wherein said cells are mammalian cells.

70. (New) A method of claim 63, wherein said cells further comprise a reporter gene construct operably linked to a transcriptional regulatory element responsive to *hedgehog* signaling, and said detectable response comprises detecting the level of expression of said reporter gene, and comparing the response of said cells to a test agent.

71. (New) A method of claim 70, wherein expression of the reporter gene is detected by determining the protein product encoded by the reporter gene.

72. (New) A method of claim 71, wherein the reporter gene product is detected by an intrinsic activity associated with that product.

73. (New) A method of claim 71, wherein the reporter gene product is detected by an enzymatic activity associated with that product.

74. (New) A method of claim 63 or 70, wherein the transcriptional regulatory element are derived from target genes selected from the group consisting of *GLI*, *patched*, *cubitus interruptus*, and *fused*.

75. (New) A method of claim 63 or 70, wherein the detectable response comprises expression of homeobox genes.

76. (New) A method of claim 75, wherein the homeobox gene is Hoxd.
77. (New) An assay of claim 30, wherein the assay detects test compounds that mimic *hedgehog*-dependent *patched* signal transduction.

The claims presented above incorporate changes as indicated by the marked-up versions below.

1. (Amended) An assay for identifying compounds that mimic the bioactivity of a having potential *hedgehog* protein bioactivity, comprising:
- (a) forming a reaction mixture including:
- (i) ~~a *hedgehog* polypeptide~~;
 - (ii) a naturally occurring *patched* receptor polypeptide; and
 - (iii) a test compound; and
- (b) detecting ~~interaction~~ activation of the *hedgehog* pathway ~~and *patched* polypeptides~~;
- wherein a statistically significant change in the ~~interaction~~ activation of the *hedgehog* pathway ~~and *patched* polypeptides~~ in the presence of the test compound, relative to the interaction activation in the absence of the test compound, indicates a ~~potential *hedgehog*-mimicking~~ activity for the test compound.
3. (Amended) The assay of claim 1, wherein the reaction mixture comprises a ~~recombinant~~ cell including a heterologous nucleic acid recombinantly expressing the *patched* receptor polypeptide.
4. (Amended) The assay of claim 1, wherein ~~the step of detecting interaction activation of the *hedgehog* and *patched* polypeptides pathway~~ comprises observing a phenotype of a cell in the presence and absence of the test compound ~~a competitive binding assay~~.
5. (Amended) The assay of claim 3, wherein ~~the step of detecting interaction activation of the *hedgehog* and *patched* polypeptides pathway~~ comprises detecting a change in the level of an intracellular second messenger responsive to signaling by the *patched* polypeptide.

6. (Amended) The assay of claim 3, wherein ~~the step of detecting interaction~~ activation of the *hedgehog* and ~~*patched* polypeptides~~ pathway comprises detecting a change in the level of expression of a gene controlled by a transcriptional regulatory sequence responsive to signaling by the *patched* polypeptide.

7. (Amended) The assay of claim 3, wherein the recombinant cell substantially lacks expression of an endogenous *patched* ~~protein~~ receptor.

17. (Amended) An assay for screening test compounds to identify agents ~~which modulate the binding that mimic a bioactivity of a~~ *hedgehog* proteins ~~with a~~ *hedgehog* receptor, comprising:

- providing a cell expressing a *hedgehog* receptor, wherein said *hedgehog* receptor binds a naturally occurring *hedgehog* polypeptide;
- contacting the cell with ~~a *hedgehog* polypeptide and~~ a test compound; and
- detecting ~~interaction~~ activation of the ~~*hedgehog* polypeptide and~~ *hedgehog* receptor, wherein a statistically significant change in the level of ~~interaction~~ activation of the *hedgehog* ~~polypeptide and~~ *hedgehog* receptor is indicative of an agent that ~~modulates the interaction of the~~ mimics a bioactivity of a *hedgehog* proteins ~~with a~~ *hedgehog* receptor.

18. (Amended) The assay of claim 17, wherein the ~~interaction~~ activation of the *hedgehog* ~~polypeptide and~~ receptor is detected by detecting a change in phenotype of the cell relative to in the absence of the test compound.

19. The assay of claim 17, wherein the change in phenotype is detected by detecting gain or loss of expression of a cell-type specific marker.

20. (Amended) The assay of claim 17, wherein the receptor transduces a signal in the cell which is sensitive to *hedgehog* binding, and the cell further comprises a reporter gene construct comprising a reporter gene in operable linkage with a transcriptional regulatory sequence sensitive to intracellular signals transduced by ~~interaction~~ activation of the *hedgehog* ~~polypeptide and~~ receptor, expression of the reporter gene providing a detectable signal for detecting ~~interaction~~ activation of the *hedgehog* ~~polypeptide and~~ receptor.

21. (Amended) The assay of ~~any of~~ claims 20 or 33, wherein the reporter gene encodes a gene product that gives rise to a detectable signal selected from ~~the group consisting of~~ color, fluorescence, luminescence, cell viability, relief of a cell nutritional requirement, cell growth, and drug resistance.
22. (Amended) The assay of claim 21, wherein the reporter gene encodes a gene product selected from ~~the group consisting of~~ chloramphenicol acetyl transferase, luciferase, betagalactosidase, and alkaline phosphatase.
23. (Amended) The assay of claim 20, wherein the reporter gene includes a transcriptional regulatory sequence of a gene selected from ~~the group consisting of~~ a *GLI* gene and *patched* gene.
24. (Amended) The assay of claim 17, wherein the receptor transduces a signal in the cell which is sensitive to *hedgehog* binding, and ~~interaction~~ activation of the *hedgehog* ~~polypeptide~~ and receptor ~~are~~ is detected by detecting change in ~~the~~ a level of an intracellular second messenger responsive to signaling by the receptor.
25. (Amended) The assay of claim 24, wherein the ~~interaction~~ activation of the *hedgehog* ~~polypeptide and~~ receptor is detected by changes in intracellular protein phosphorylation.
26. The assay of claim 17, wherein the receptor is a *patched* receptor.
27. (Amended) The assay of ~~any of~~ claim 17 ~~and~~ or 26, wherein the cell further comprises a heterologous gene construct encoding the receptor.
29. The assay of claim 17, wherein the cell further comprises one or more heterologous gene constructs encoding *costal-2*, *fused* and/or *smoothened* genes, or homologs thereof.
30. (Amended) An assay for screening test compounds to identify agents ~~which modulate the activity of~~ that activate a naturally occurring mammalian *patched* receptor ~~protein~~, comprising:

- i. providing a cell ~~expressing~~ having a recombinant expression vector encoding a naturally occurring mammalian patched receptor protein;
- ii. contacting the cell with a test compound under conditions whereunder the patched protein is expressed; and
- iii. detecting an effect, if any, of the test compound on signal transduction by the patched protein,

wherein a statistically significant change in the signal transduction of the patched receptor in the presence of the test compound, relative to in the absence of the test compound or ~~absence~~ of the patched receptor protein, is indicative of an agent that modulates the activity of patched receptor protein.

31. (Amended) The assay of claim 30, wherein the signal transduction by the patched receptor protein is detected by detecting a change in phenotype of the cell relative to in the absence of the test compound.

32. (Amended) The assay of claim 30, wherein the ~~patched protein is recombinantly expressed in the cell~~ is a human cell.

33. (Amended) The assay of claim 30, wherein the cell further comprises a reporter gene construct comprising a reporter gene in operable linkage with a transcriptional regulatory sequence sensitive to intracellular signals transduced by interaction of a hedgehog polypeptide with the patched receptor protein, expression of the reporter gene providing a detectable signal for detecting signal transduction by the patched receptor protein.

34. (Amended) The assay of ~~any of~~ claims 1, 15, 26 or 30, wherein the patched receptor polypeptide is of vertebrate origin.

35. (Amended) The assay of claim 34, wherein the patched receptor polypeptide is of a mammalian patched receptor origin.

36. (Amended) The assay of claim 35, wherein the patched receptor polypeptide is a human patched protein receptor.

42. (Amended) The assay of any of claims 3, 17 or 30, wherein the ~~recombinant~~ cell is a metazoan cell.
43. (Amended) The assay of claim 42, wherein the ~~recombinant~~ cell is a mammalian cell.
44. (Amended) The assay of claim 42, wherein the ~~recombinant~~ cell is an insect cell.
45. (Amended) The assay of any of claims 3, 17, or 30, wherein the ~~recombinant~~ cell is a oocyte.
46. (Amended) The assay of any of claims 3, 17, or 30, wherein the ~~recombinant~~ cell is a yeast cell.
47. (Amended) The assay of ~~any of~~ claims 1, 8, 17, or 30, wherein the steps of the assay are repeated for a variegated library of at least 100 different test compounds.
48. (Amended) The assay of ~~any of~~ claims 1, 8, 17 or 30, wherein the test compound is selected from ~~the group consisting of~~ small organic molecules, and natural product extracts.
49. (Amended) The assay of ~~any of~~ claims 1, 8, 17 or 30, further comprising ~~a step of~~ preparing a pharmaceutical preparation of one or more compounds identified.